

## Bioleaching of metals from secondary materials using glycolipid biosurfactants

Martijn Castelein<sup>a</sup>, Florian Verbruggen<sup>b</sup>, Lisa Van Renterghem<sup>a</sup>, Jeroen Spooren<sup>c</sup>,  
Lourdes Yurramendi<sup>d</sup>, Gijs Du Laing<sup>e</sup>, Nico Boon<sup>b</sup>, Wim Soetaert<sup>a,f</sup>, Tom Hennebel<sup>b</sup>,  
Sophie Roelants<sup>a,f,\*</sup>, Adam J. Williamson<sup>b,\*</sup>

<sup>a</sup> Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Department of Biotechnology, Faculty of Bioscience Engineering Ghent University, Coupure Links 653, 9000 Ghent, Belgium

<sup>b</sup> Center for Microbial Ecology and Technology (CMET), Department of Biotechnology, Faculty of Bioscience Engineering Ghent University, Coupure Links 653, 9000 Ghent, Belgium

<sup>c</sup> Waste Recycling Technologies, Flemish Institute for Technological Research, VITO N. V. Boerentang 200, 2400 Mol, Belgium

<sup>d</sup> TECNALIA, Energy and Environment Division, Mikeletegi Pasealekua 2, E-20009 Donostia-San Sebastián - Gipuzkoa, Spain

<sup>e</sup> Laboratory of Analytical Chemistry and Applied Ecochemistry (ECOCHM), Department of Green Chemistry and Technology, Faculty of Bioscience Engineering Ghent University, Coupure Links 653, 9000 Ghent, Belgium

<sup>f</sup> Bio Base Europe Pilot Plant, Rodenhuzekaai 1, 9042 Ghent - Desteldonk, Belgium

### ARTICLE INFO

#### Keywords:

Sophorolipids  
Bioleaching  
Heavy metal recovery  
Fayalite  
Copper

### ABSTRACT

With the global demand for economically important metals increasing, compounded by the depletion of readily accessible ores, secondary resources and low-grade ores are being targeted to meet growing demands. Novel technologies developed within biobased industries, such as microbial biosurfactants, could be implemented to improve the sustainability of traditional hydrometallurgy techniques. This study investigates newly developed microbial biosurfactants (acidic- and bolaform glycolipids) for the leaching of metals (particularly Cu and Zn) from a suite of mine tailings, metallurgical sludges and automotive shredder residues. Generally, acidic sophorolipids were the most performant, and optimal Cu leaching was observed from a fayalite slag (27%) and a copper sulfide mine tailing (53%). Further investigation of the leached fayalite material showed that leaching was occurring from small metallic Cu droplets in this material via a corrosion-based mechanism, and/or from Cu-Pb sulfides, selective against dominant Fe-silicate matrices. This study highlights that acidic sophorolipid microbial biosurfactants have the potential to leach Cu and Zn from low-grade secondary materials. It also provides important fundamental insights into biosurfactant-metal and mineral interactions that are currently unexplored. Together, the convergence of leaching and mining industries with bio-industries can improve material recovery and will positively impact the bio- and circular economies and the environment.

### 1. Introduction

The global demand for economically important metals, such as copper (31.97 Mt.y<sup>-1</sup> expected in 2030) and zinc (22.19 Mt.y<sup>-1</sup> expected in 2030) (Rietveld et al., 2018), is on the rise and directly proportional to the growth of a broad spectrum of industries, supported by rapidly developing renewable energy, electronics and (electric) vehicle technologies (Guo et al., 2009; Weber and Rutula, 2001; Nessa and Khan, 2016; Sethurajan and van Hullebusch, 2019). In contrast, the decrease in easily accessible ores (Desjardins, 2019) and large environmental

footprint of mining activities has led to calls for more sustainable technologies for metal extraction and recovery from existing industrial residues, tailings and slags (Ardau et al., 2009; Sethurajan et al., 2018; Potysz et al., 2018; Piatak, 2018). Although these secondary resources are generally lower in metal content than their respective ores, their volumes are enormous (Alloway, 2012) with mill tailings from Cu, Fe, Pb and Zn mining already surpassing 500 Mt.y<sup>-1</sup> in Europe alone (Kurylak et al., 2016). For example, with a global Cu production of 21 Mt.y<sup>-1</sup> (2018) largely from primary ores comes a vast amount of Cu containing fayalite slags (Garside, 2019). By 2003, the global annual fayalite slag

\* Corresponding authors.

E-mail addresses: [Sophie.roelants@ugent.be](mailto:Sophie.roelants@ugent.be) (S. Roelants), [williams@cenbg.in2p3.fr](mailto:williams@cenbg.in2p3.fr) (A.J. Williamson).

<https://doi.org/10.1016/j.mineng.2020.106665>

Received 27 May 2020; Received in revised form 29 September 2020; Accepted 7 October 2020

0892-6875/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

production surpassed 30 Mt.y<sup>-1</sup>, (Gorai and Jana, 2003) with almost 3 Mt.y<sup>-1</sup> produced in Europe. The estimated total fayalite slag production over the period of 1900–2004 was 842 Mt (Sudbury et al., 2009), with an approximate average Cu composition of 1% (Hunt et al., 2013). Still, many of these metal containing waste streams are landfilled, which can lead to the dissolution of heavy metals and other contaminants in surface and groundwaters (Piatak, 2018; Bouzayani et al., 2014; Ettler, 2016). Alternate routes for these secondary residues are currently limited to immobilization in concrete, or incineration and compaction to reduce the volume. The result is a high cost for containment of possible hazards, significant land occupation and associated cost for sanitation if sinks are not managed well (Alloway, 2012).

Conventional hydrometallurgy routes for the extraction and concentration of metals from primary ores involve froth flotation and leaching technologies which typically employ strong acids, bases, solvents and/or surfactants (Michaud, 2019). Whilst enabling favorable high and selective metal extractions for high grade primary ores, problems of toxicity and pollution, handling and loss of lixiviant through matrix interactions limit their sustainability for lower grade secondary materials (Hoque and Philip, 2011). Moreover, the surfactants that are currently being used in metal extraction applications (Mujicic and Coleman, 2016; Seelmann-eggert et al., 2006) are mostly fossil based and/or produced through chemical processes, all giving drawbacks in terms of sustainability. Biohydrometallurgy implements microbially produced lixiviants for metal extraction and/or recovery and has been implemented on an industrial scale for Cu and Au heap leaching via autotrophic pathways (Ardau et al., 2009; Sethurajan et al., 2018; Muravyov et al., 2012; Johnson, 2014). However, autotrophic leaching technologies which make use of acidophilic microorganisms, are limited to sulfidic matrices and thus cannot exploit the growing heterogeneous secondary source inventory (Natarajan, 2018).

Biomolecules such as organic – and nucleic acids, extracellular polymeric substances (EPS) and (microbial) biosurfactants are attracting interest in this field (Pollmann et al., 2018) due to their non-requirement for sulfidic matrices, stability during use (for reuse and recovery) and biodegradability after use. Biosurfactants are 100% biomass sourced surfactants while microbial biosurfactants are produced through a biological production process applying microorganisms as production hosts. The rise of the bio-economy has led to increased production volumes, with biosurfactants expecting to reach 462 kT by 2020 (Grand View Research, 2018) and microbial biosurfactants expected to increase dramatically the following years (Markets and markets, 2017). The resulting decreasing production costs, through the economy of scale (Van Renterghem et al., 2018), will facilitate the implementation of (microbial) biosurfactants towards new applications with high potential, including biohydrometallurgy.

Surfactants and (microbial) biosurfactants have shown potential for the removal of (heavy metal) contaminants from soils through soil washing, remediation or sludge dredging (Schippers et al., 2000; Tandy et al., 2004; Kim et al., 2016; Mulligan et al., 2001; Yang et al., 2016; Mulligan and Wang, 2006). However, only a select number of studies have investigated the use of biosurfactants for metal recovery from pure minerals (quartz, clays) (Massara et al., 2007; Aşçi et al., 2010), sediments (Dahrazma and Mulligan, 2007) and industrial wastes (Franzetti et al., 2014; Diaz et al., 2013). Moreover, nearly all studies to date have focused on the microbial biosurfactant rhamnolipids, with only one study to date investigating the use of the microbial biosurfactant sophorolipids (SLs) for metal extraction, despite being already applied in other domains at industrial scale (Solaiman et al., 2018). Sophorolipids are surface-active glycolipids composed out of a disaccharide sophorose glycosidically bound to a hydroxylated fatty acid (Gorin et al., 1961) and naturally occur in a mixture of open (acidic sophorolipids) and closed (lactonic sophorolipids) forms. Moreover, new types of sophorolipids, sophorosides (SSs) and glucosides with promising metal coordination properties have been developed (Van Renterghem et al., 2020), including innovative bolaform sophorolipids and sophorosides (Price

et al., 2012; Van Renterghem et al., 2018). The latter consist of two sophorose units linked by a hydrophobic linker, resulting in bolaform structures (Fuhrhop and Wang, 2004), which has been correlated with improved supramolecular assembly (Baccile et al., 2016a; Baccile et al., 2012) behaviour. As other bola-amphiphiles have been described to exhibit decreased permeability compared to regular polar lipids (Fuhrhop and Wang, 2004; Puri et al., 2009), these physiochemical traits may improve metal extraction and coordination stability over other (bio) surfactants, yet remain unexplored in the context of biohydrometallurgy.

Multiple mechanisms have been attributed to (bio)surfactant-mineral interactions. Surfactant-surface interactions can favor metal mobility through a reduction of interfacial tension and/or an increase in mineral wettability by changing the apparent hydrophilicity of the material (Michaud, 2019). The strength of the biosurfactant-metal complex can be influenced by electrostatic Van der Waals forces, which can result in the formation of soluble complexes, incorporation of metals into micelles or other supramolecular structures depending on the surfactant type (Van Renterghem et al., 2019; Baccile et al., 2016a, 2016b, 2017, 2019; Dhasaiyan et al., 2018; Peyre et al., 2017; Dhasaiyan et al., 2017; Sarubbo et al., 2015). The current generally accepted model for metal extraction by (bio)surfactants is based on metal incorporation into (bio)surfactant micelles, involving adsorption, biosurfactant-metal complex formation, desorption and possible micelle inclusion (Mulligan et al., 2001; Sarubbo et al., 2015; Rufino et al., 2012; Luna et al., 2016; Mulligan et al., 1999). However, it is yet to be seen if a similar model exists for other (microbial) biosurfactants and more complex materials such as metallurgical residues.

The objective of this study is to assess the potential for emerging sophorolipid and sophoroside type microbial biosurfactants to extract metals from secondary sources. Moreover, we aim to provide useful insights into surfactant-metal interactions for (bolaform) sophorolipid and -sophoroside glycolipids that are currently largely unknown. By modifying the biosurfactant type and concentration, the lixiviant pH and the time of leaching, optimal combinations of biosurfactants, leaching parameters and secondary materials could be obtained. Further underlying mechanisms and mineralogical characterisations were then pursued on selected materials to complement the original screening of materials and biosurfactants and to give prospective towards future bioleaching studies and technologies using biosurfactants.

## 2. Materials and methods

### 2.1. Materials and leaching experiments

Leaching experiments and SEM analyses were performed on ten different materials allocated to four types of materials (SI Table 1, SI Figs. 1-7): zinc processing sludges and slags (fayalite slag, iron-rich zinc processing sludge and zinc processing filter-press sludge), copper sulfide tailings (low-grade CuS tailing and high-grade CuS tailing), shredder and sludges from end of life vehicles (shredder sludge filter cake, low-grade shredder residue and high-grade shredder residue) and stainless steel residues (zinc-rich lime sludge and chromium-nickel-rich filter cake sludge). Apart from the low-grade CuS tailing, the shredder sludge filter cake and the low-grade shredder residue, all materials have already been reported (Williamson et al., 2021) and are briefly described in this paper (SI Table 1). All materials have been prepared by drying (72 h, 70 °C) followed by sieving to retrieve particles with a maximal diameter of 1 mm without determination of particle size distribution. Pseudo-total metal content was determined through aqua regia digestion of 1 g of each material.

### 2.2. Strains and culture conditions used for glycolipid production

Seven different sophorolipids (SLs) and sophorosides (SSs) belonging to three types of glycolipids/glycosides were evaluated for their leaching

potential: acidic SLs (non-acetylated, medium degree acetylated and di-acetylated acidic SL, Fig. 1-A), glucolipids (non-acetylated acidic glucolipid, Fig. 1-B), bolaform SL (acetylated bolaform SL, Fig. 1-C) and bolaform SSs (non-acetylated bolaform SS and acetylated bolaform SS, Fig. 1-D). A structural overview is given in Fig. 1 and detailed physicochemical properties (molecular mass, fatty acid chain, purity, uniformity, CMC, solubility and surface tension) can be found in SI Table 2. Synthesis of the different (acetylated) sophorolipids/sophorosides has been previously reported through aerobic fermentation with different strains of the yeast *Starmerella bombicola* as shown in SI Table 2. Absence of residual metal ions in the biosurfactant batches was confirmed through Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES, Varian Vista MPX, US) analysis.

### 2.3. Leaching experiments

An initial bio-leaching screening was performed in single batch for all combinations of material and biosurfactant, after which further optimization and characterization experiments were done in triplicate. Bioleaching was executed in horizontally sealed closed test tubes (50 mL) at a shaking speed of 120 rpm and in temperature controlled conditions (30 °C). All experiments were performed at a 1:10 solid to liquid ratio (1 g slag/ 10 mL biosurfactant solution) with a headspace of 40 mL to maintain aerobic conditions. The biosurfactant concentration in the leachate was chosen at 1% (10 g.L<sup>-1</sup>), except for the concentration optimizations that were executed at 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1 and 2.5%. A leaching time of 7 days was selected for all leaching experiments, supplemented with data for shorter periods (1, 4, 24, 48 h) during kinetic optimization experiments. The pH was adjusted with NaOH or HCl during pH-controlled experiments. Control leaching experiments were performed using MilliQ (MQ) dH<sub>2</sub>O instead of the biosurfactant solution. Leaching of metals is either expressed as yield (Y, mg metal leached per gram of material added) or as efficiency (E, the percentage removal of the original metal content).

### 2.4. Geochemical analyses

The pH was measured using a Consort multiparameter analyser C3020. Total digestions and biosurfactant leachates were filtered through a 0.2 µm filter and diluted in 1% HNO<sub>3</sub> prior to analysis by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES, Varian Vista MPX, US) for Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and Ca. All samples were diluted using 1% HNO<sub>3</sub> prior to analysis to match the concentration range. Quantitative determination of the glycolipid

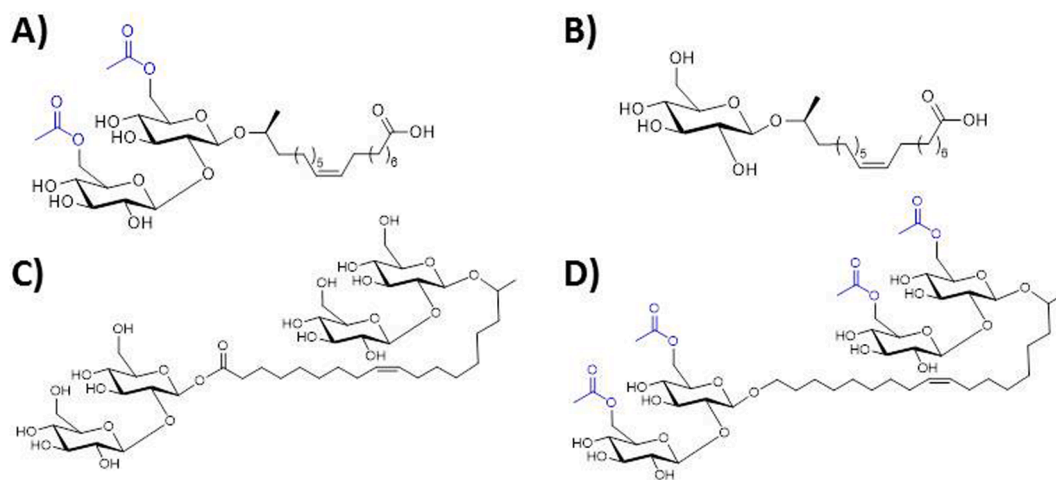
concentration was determined through Ultrahigh Pressure Liquid Chromatography —Evaporative Light Scattering Detector (UPLC-ELSD) analysis (Waters Acquity H-Class ULC, Waters Acquity ELSD) using an Acquity UPLC CSH C18 column (130 Å, 1.7 µm, 2.1 mm × 50 mm) (Waters) and a gradient elution system based on 0.5% acetic acid in MQ dH<sub>2</sub>O (A) and 100% acetonitrile (B) at a flow rate of 0.6 mL.min<sup>-1</sup> (linear increase from 5% A to 95% A in 6.78 min and a linear decrease back to 5% A by 8.42 min). Eh-pH diagrams of Cu and Zn were used to rationalize the leaching behavior in the experimental conditions and were calculated with the chemical equilibrium diagram software Hydra/Medusa.

### 2.5. Mineralogical analyses

For the starting materials of the fayalite, Zn-rich lime sludge, Fe-rich Zn processing residue and shredder residues, SEM analysis was carried out by a FEI NOVA NANOSEM 450 with EDX analyser BRUKER QUANTAX 200 with silicon drift detector (SDD) for which samples were imbedded in an epoxy resin that was subsequently polished. For the low and high-grade CuS tailings and the fayalite material time course experiment, SEM was performed using a Jeol SM-5910LV. The images were taken at high vacuum mode with 20 kV voltage in a solid-state backscatter detector (SSD-BSD). Oxford instrument INCAx-act Energy Dispersive X-ray detector (EDS) was used for chemical semi-quantitative analysis. For the sample preparation, the sample was embedded under vacuum conditions in epoxy resin, pre-polished with abrasive powder and coated with a thin layer of graphite. Samples were embedded in an epoxy resin, which was subsequently polished. For this operation, solvents and resins were selected depending on the presence of soluble mineral phases in the materials.

### 2.6. Corrosion experiments

Electrochemical measurements were carried out in a three-electrode cell with a volume of 50 mL (T = 21 °C). 1 cm of a copper wire working electrode (φ = 1.35 mm) with a projected surface area of 0.91 cm<sup>2</sup> was exposed to the electrolyte, while a platinum spiral wire (10 cm) was used as the counter electrode. The copper wire was polished in different steps with SiC papers up to grit 4000 (Struers GmbH, The Netherlands). Reported potentials refer to the Ag/AgCl reference electrode (3 M KCl, ALS, Japan, + 0.203 V vs. standard hydrogen electrode at 21 °C) and electrochemical data were recorded with a potentiostat VSP-300 (Bio-logic SAS, France) via the EC-lab software. The experiments were conducted in a 0.1 M acetate buffered solution at pH 4.7 while the solution was well



**Fig. 1.** A structural overview of used sophorolipid and sophoroside microbial biosurfactants. In blue, possible acetylations are marked. A) C18:1 acidic sophorolipid (nAc ASL, mAc ASL, dAc ASL). B) C18:1 non-acetylated glucolipid (nAc AGL). C) C18:1 acetylated bola sophorolipid (nAc BSL). D) C18:1 bola sophoroside (nAc BSS, Ac BSS).

stirred at 500 rpm. To study the effect of the non-acetylated acidic sophorolipid on the copper dissolution reaction cyclic voltammetry was performed. The voltammographic cycles started after 10 min of Open Circuit Potential (OCP) mode and were scanned from  $-0.05$  V vs OCP to  $+0.10$  V vs OCP for 3 times for response stabilization with a scan rate of  $5 \text{ mV.s}^{-1}$ . From this data, the corrosion potential ( $E_{\text{corr}}$ ) and corrosion current ( $I_{\text{corr}}$ ) were extracted with Tafel fit tool (EC-lab software) for the different non-acetylated acidic sophorolipid concentrations.

### 3. Results and discussion

#### 3.1. Screening of secondary materials using biosurfactants

To identify the biosurfactant type and material combinations that showed promising leaching efficiencies for target metals, a singular bioleaching screening of ten materials with seven distinct biosurfactants at a concentration of  $10 \text{ g.L}^{-1}$  was conducted for one week (Fig. 2, SI Table 1, SI Table 2, SI Table 3). The glucolipid and the di-acetylated acidic sophorolipid had a low solubility, particularly at low pH ( $1 \text{ g.L}^{-1}$  and  $0.08 \text{ g.L}^{-1}$  at pH 2 respectively). The subsequent loss of soluble lixiviant was the likely cause of the low leaching for all materials, thus no further exploration of these biosurfactants was pursued. Poor metal

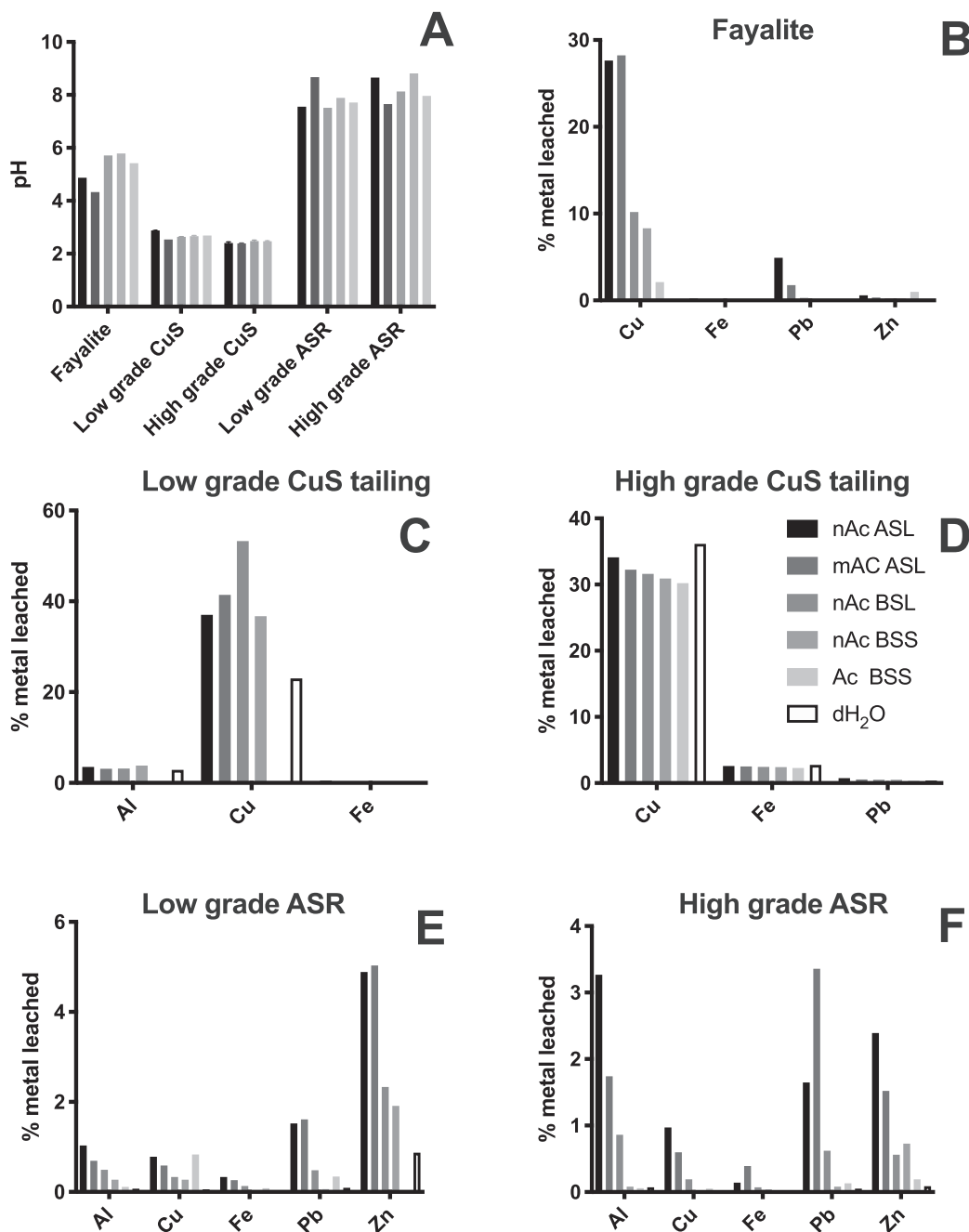


Fig. 2. pH (A) and metal leaching from the fayalite (B), low (C) and high (D) grade copper sulphide (CuS) tailing and low (E) and high (F) grade automotive shredder residues (ASRs) after a contact time of 7 days with 5 biosurfactants compared to the MQ dH<sub>2</sub>O water control. Bars represent a single sample.

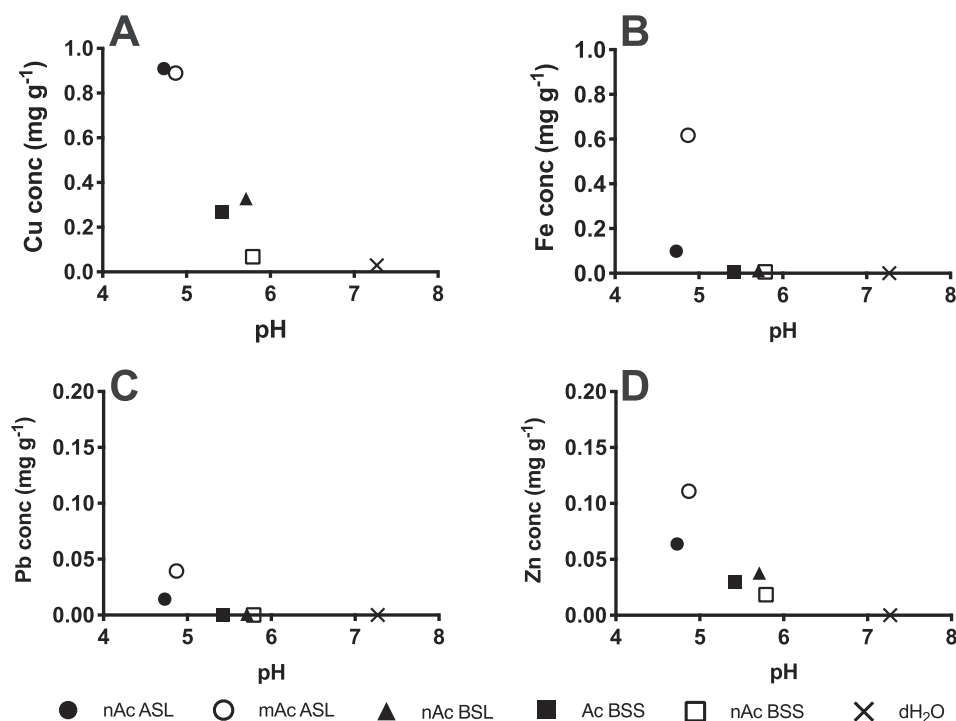
leaching ( $E < 1\%$  for all metals) was observed for the Fe-rich Zn processing sludge, the Zn processing filter press sludge, the shredder sludge filter cake, the Zn-rich lime sludge and the Cr-Ni rich filter cake sludge, highlighting the unsuitability of the use of these biosurfactants to leach metals from these materials (SI Table 3). For the Zn-rich lime sludge and the Zn processing filter press sludge, the pH buffered to weakly alkaline conditions (10.5–12.0), limiting  $Zn^{2+}$  solubility (SI Fig. 8-A). For the other materials that all buffered at neutral pH, it could represent the recalcitrant target metal mineralogy, with Cu (SI Table 1, SI Fig. 8-B, C and D), Zn and Ni predominantly in iron spinel phases, franklinite and trevorite, compounded by their low solubility in the pH ranges of these experiments (SI Fig. 9).

For the remaining five materials (fayalite, automotive shredder residues (ASRs) and copper sulfide (CuS) tailings), Cu was the highest extracted metal, with optimal extraction efficiencies from the fayalite slag ( $E = 2.12$ – $27.63\%$ ) and the low-grade CuS tailing ( $E = 36.73$ – $53.30\%$ ), where Cu was present in metallic or sulfidic form (SI Table 1, Fig. 2 and SI Fig. 1-A, B and 3). The ASRs also were primarily comprised of metallic Cu(0) (SI Table 1), and whilst similar leached Cu concentrations were observed (up to  $1.5 \text{ mg.g}^{-1}$ ), this represented a much lower leaching efficiency ( $E < 1\%$ , Fig. 2). With respect to other metals, typically lower but measurable leaching ( $Y > 0.1 \text{ mg.g}^{-1}$ ) of Zn, Pb and Al was generally observed (Fig. 2). The overall lower extraction yields of these metals in the materials are likely a result of the lower relative abundance of extractable (i.e. sulfide,  $M(O)$ ) against more recalcitrant phases such as spinel (e.g. franklinite ( $ZnFe_2O_4$ )) or amorphous ( $Pb/ZnSO_4$ , calcium gordaite  $CaZn_8(SO_4)_2Cl_2(OH)$ , (SI Table 1)) phases that are not expected to be affected easily by weak acids and biosurfactants (Aldrian et al., 2015; Mardones, 2012). Fortuitously, low leaching yields and efficiencies of Fe ( $E < 2.57\%$  for the high grade CuS tailing,  $E < 1\%$  for all materials), which was typically the highest competing cation by mass, were observed (Fig. 2). However, poor leaching ( $Y < 0.1 \text{ mg.g}^{-1}$ ) or no difference compared to the blank was observed for all other metals analysed (i.e. Cd, Co, Cr, Mn and Ni),

highlighting unsuitability for application of biosurfactants for these metal-mineral combinations.

In general, both the non- and medium degree acetylated acidic sophorolipids were the most performant metal extracting biosurfactants that buffered the leachate to lower pH values (Fig. 2 and Fig. 3). No distinct impact of acetylation was observed between these biosurfactants however. The pH evolved in the pregnant leaching solutions is a complex interplay of biosurfactant chemistry and material mineralogy, yet a correlation between pH, surfactant type and metal leached became apparent, illustrated for the fayalite slag (Fig. 3). For the low and high-grade CuS tailings that all buffered to a similar low pH ( $\sim 3$ ), the added impact of the acidic sophorolipids was less noticeable. For the high-grade CuS tailing, little difference between all surfactants was observed for Cu extraction and similar to the  $dH_2O$  control ( $E = 30.25$ – $36.14\%$ ). Interestingly, for the low-grade CuS tailing, the bola sophorolipid was the most performant lixiviant ( $Y = 0.23 \text{ mg.g}^{-1}$ ,  $E = 53.30\%$  of Cu leached versus  $E = 23.04\%$  for the control) and recent work has demonstrated enhanced CuS oxidation by bola sophorolipids (Dhar et al., 2021).

Sustainable metal extraction is reliant on both a high selectivity to reduce further processing costs and minimal loss of lixiviant for potential reuse. Whilst the acetylated acidic sophorolipid was 5.8 times more selective towards Cu over Fe than the non-acetylated form in the fayalite material, it was slightly more susceptible to co-precipitation and/or irreversible adsorption (resp. 18% loss vs. 12%). Considering the high Cu leaching efficiency and the moderate yield of both acidic sophorolipid biosurfactants towards the fayalite and the limited expected loss of surfactant to the material, compounded by its favourable chemistry (practical handling of the product, carboxylic functionality and high solubility over a large pH range), the non-acetylated acidic sophorolipid was chosen for further investigation with the fayalite material.



**Fig. 3.** A scatter plot of pH vs the leaching efficiency yield of Cu (A), Fe (B), Pb (C) and Zn (D) from the fayalite slag for acidic sophorolipids (circles), bola sophorolipids (squares), bola sophorolipid (triangles) and control with  $dH_2O$  (cross) after a contact time of seven days. Closed shapes represent acetylated forms and open shapes represent non-acetylated forms. The average value and standard deviation of  $N = 3$  replicates is plotted. Error bars are not visible due to being smaller than symbol.



### 3.2. Mechanisms behind metal leaching from the fayalite

To further explore the mechanistic principles that are involved in the bioleaching process, biosurfactant leaching experiments were performed with the non-acetylated acidic sophorolipid and the fayalite material over a controlled pH range of 3–11 (Fig. 4) and sampled periodically over seven days in an unbuffered system (Fig. 5). In the HCl/NaOH pH-controlled experiment, an increased leaching efficiency of Cu, Fe, Pb and Zn in comparison with the non-surfactant control was observed at all pH values, with a convergence between inorganic acid leaching and the surfactant only seen for Cu at pH 3. These results highlight the added impact of the biosurfactant on metal extraction from the fayalite material, supporting earlier observations from the material screening. Previous work has implicated metal stabilization in micelles (Mulligan et al., 2001; Sarubbo et al., 2015), thus a similar mechanism could be occurring for the fayalite in contact with the non-acetylated acidic sophorolipid.

To give further insights into temporal Cu, Fe, Zn and Pb leaching from the fayalite slag, a kinetic study was performed over one week. More than half of the total copper extracted was leached from the fayalite material with the non-acetylated acidic sophorolipid within four hours (Fig. 5-A), followed by a lower rate of leaching over the next seven days. A similar trend was observed for Fe, Zn and Pb, (Fig. 5-A and B). Given the pKa of acidic sophorolipids is 6.1 (SI Fig. 10), it is unlikely that the plateau in Cu leaching was a result of loss of protonation over this half unit pH shift from  $4.37 \pm 0.1$  to  $4.86 \pm 0.1$  by day 7 (Fig. 5-C). A simple thermodynamic model of the system at  $16 \mu\text{mol.L}^{-1}$ ,  $160 \mu\text{mol.L}^{-1}$  and  $1.6 \text{ mmol.L}^{-1} \text{ Cu}^{2+}$  shows that the pH boundary of CuO precipitation shifts from pH  $\sim 6.5$  to  $\sim 5.5$  (SI Fig. 8-B, C and D), thus rules out the insolubility of CuO phases over the concentration ranges measured in these experiments. This plateau thus represents a limitation of lixiviant and/or biosurfactant accessible mineral phases (i.e. Cu(0) or mixed CuS sulfides). A high degree of sorption of non-acetylated acidic sophorolipid to the fayalite material

occurred within 1 h ( $30.6 \pm 2.3\%$  of total biosurfactant added), followed by a (slower) release up to 2 days, with  $87.1 \pm 2.1\%$  of the total surfactant added remaining in solution, which remained essentially constant for the further duration of the leaching experiment (Fig. 5-D). The fast sorption and subsequent desorption behavior is in agreement with previously proposed (non-ionic) biosurfactant leaching models and also potentially attributes to surface Cu-surfactant binding and release (Mulligan et al., 1999).

SEM imaging on the starting fayalite material and after early (0.5 h) and longer (7d) non-acetylated acidic sophorolipid leaching of the fayalite showed that in both the original and leached material, Al-Fe-silicates and Fe-oxides dominated, highlighting that biosurfactants did not alter the bulk mineralogy of this material (SI Fig. 1-A, Fig. 6). Zn and Pb were in smaller particles and were relatively evenly distributed across the material, thus either associated with and/or incorporated into the fayalite spinel phases and associated in mixed Pb-Cu sulfides, which were also present in larger aggregates ( $\sim 15 \mu\text{m}$ ) (Fig. 6-A). SEM imaging on these larger Cu-Pb sulfides before, during and after leaching (Fig. 6-A, B and C) showed a decrease in the copper abundance in the Cu-Pb mixed sulfides, confirming earlier findings of a selective removal of copper from these (mixed) sulfide minerals. The selective dissolution of sulfides over silicates in acidic leachates has also been demonstrated previously (Piatak, 2018; Parsons et al., 2001).

Metal extraction from sulfide minerals relies on the use of an acid and an oxidizing agent to release copper from the mineral lattice into solution. To date, no detailed mechanistic models exist to explain the increased leaching rates of copper sulfide minerals mediated by surfactants (Zhang et al., 2018). The dissolution of copper from copper sulfide minerals often is inhibited by the formation of an inert layer with sulfur containing intermediates as a result of the oxidative process (Zhang et al., 2018; Ram et al., 2020; Jorjani and Ghahreman, 2017). However, increased specific surface area (increased wettability) on the inert or passive sulfur layer seems to partially circumvent the rate limiting step (Liu et al., 2015). The increased wettability promotes

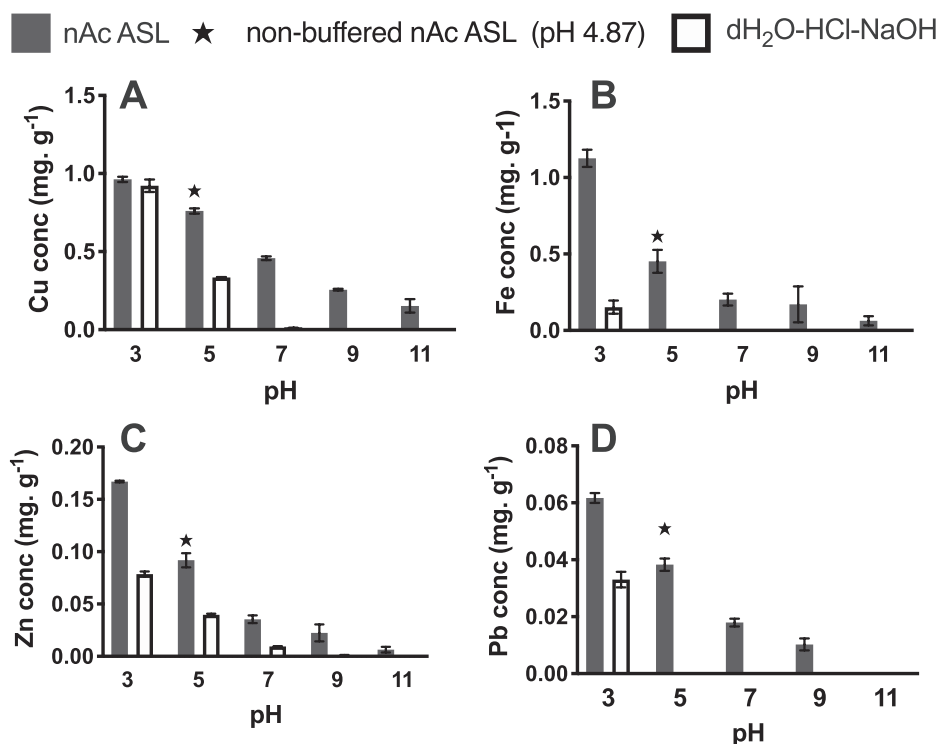
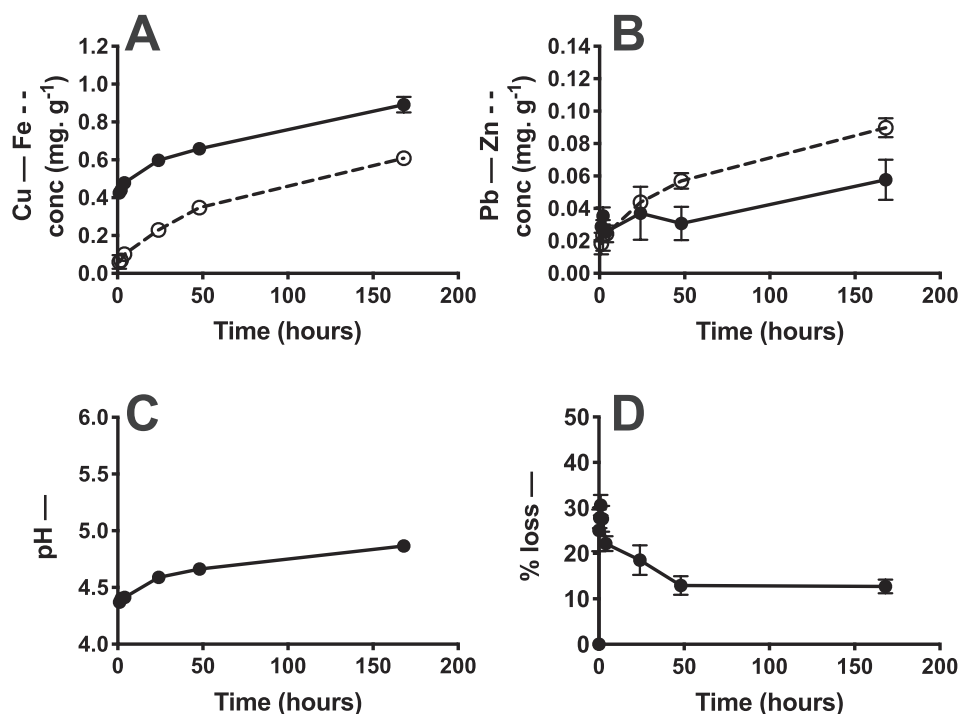


Fig. 4. A comparison of leaching efficiency for the non-acetylated acidic sophorolipid (dark grey bar) compared to a MQ dH<sub>2</sub>O control (white bar) poised at pH 3–11 for Cu (A), Fe (B), Zn (C) and Pb (D) from the fayalite slag. A star represents the non-buffered nAc ASL sample that was measured at pH 4.87. The average value and standard deviation of N = 3 replicates is plotted.



**Fig. 5.** Leaching of Cu, Fe (A), Pb and Zn (B), pH (C) and percentage of surfactant removal from solution (D), over a one week leaching experiment of the fayalite material with the non-acetylated acidic sophorolipid. The average value and standard deviation of  $N = 3$  replicates is plotted.

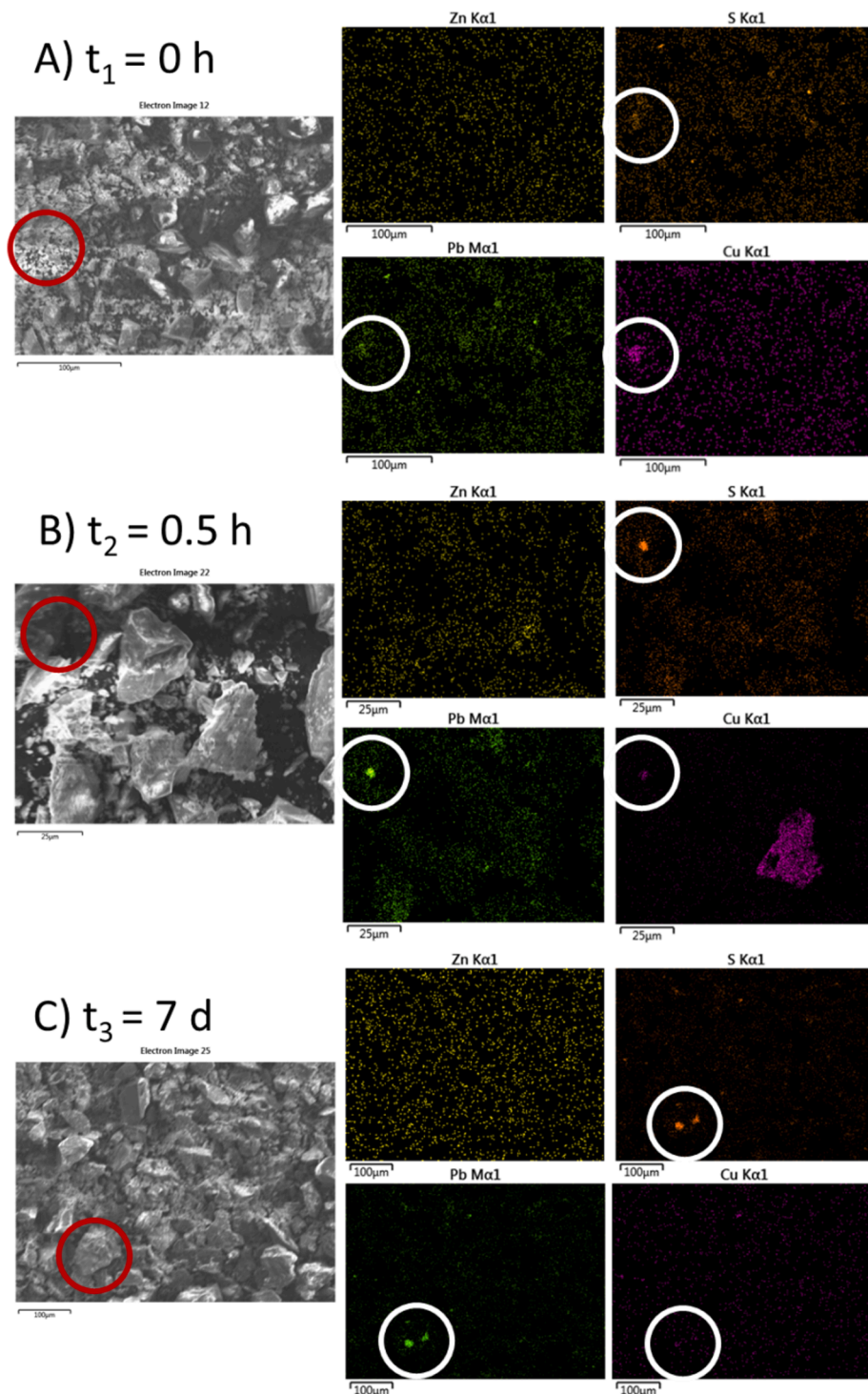
interaction between the value mineral and the chemical reagents, and hence increases the leaching rate. The combined effect of biosurfactant induced surface tension reduction and acid based electrochemical copper sulfide oxidation results in the release of  $\text{Cu}^{2+}$  into solution. Recent work has also shown the oxidation of Cu from a copper sulfide mineral dijurleite (Dhar et al., 2021), thus a similar mechanisms could be occurring in both this sample and the CuS tailings.

With the presence of metallic Cu in the fayalite slag (droplets of  $\sim 25$   $\mu\text{m}$  diameter, Fig. 6-B) and leaching of Cu from automotive shredder residues bearing exclusively Cu(0), we also explored the potential for surfactant mediated Cu(0) oxidation via corrosion. While surfactants are known to inhibit corrosion under strongly corrosive conditions (Malik et al., 2011), several studies have demonstrated enhanced corrosion by acidic compounds (Maes et al., 2017), yet no work to date has investigated these processes for acidic sophorolipids. A corrosion experiment was performed on a copper wire with increasing loadings of the non-acetylated acid sophorolipid (Fig. 7). With increasing concentration, the corrosion potential value  $E_{\text{corr}}$  decreased between 1.25 and 10  $\text{g.L}^{-1}$  surfactant, confirming an increased susceptibility of Cu(0) to oxidation and matching the observations from the earlier leaching experiments. These additional biosurfactant-surface interactions result in lowering the local surface pH and disruption of the passive layer of metallic copper, enhancing the solubilization process. The requirement for direct contact of the surfactant to the material surface could thus impede the extraction of Zn and Pb when associated with silicate mineral phases, resulting in the lower leaching yields in this study. The corrosion rate also increased up to  $18.15 \mu\text{m}.\text{year}^{-1}$  at 20  $\text{g.L}^{-1}$ , supporting the corrosion potential data (Fig. 7). A drop in the corrosion rate at higher concentrations of biosurfactant ( $>20 \text{ g.L}^{-1}$ ) supports the hypothesis towards corrosion inhibition at these high biosurfactant concentrations. Maximal limits of copper leaching from the fayalite material could thus either represent the amount of copper species susceptible to oxidation, or correspond to the maximal surface coverage of the biosurfactant at the Cu surface, highlighted during the corrosion experiment, which is an aspect for further investigation.

### 3.3. Impact of the acidic sophorolipid concentration on Cu leaching

To identify whether metal extraction was limited by biosurfactant concentration and to further explore additional biosurfactant-metal interactions, leaching experiments were set up above and below the critical micelle concentration (CMC:  $0.216 \text{ g.L}^{-1}$ ) of the non-acetylated acidic sophorolipid, from 0.1 to 25  $\text{g.L}^{-1}$  (Fig. 8). A decrease in final pH from 6.5 to 4.3 was observed with increasing biosurfactant addition (Fig. 8-A), associated with an increase in copper leaching efficiency up to 31%. Interestingly, the increase in copper leaching was not linear, but rather in three steps (Fig. 8-B). Poor leaching below  $0.5 \text{ g.L}^{-1}$  could be a result of the lack of biosurfactant micelles that sequester copper from the surface to drive the leaching process (Mulligan et al., 1999). The sharp increase between 0.5 and  $1 \text{ g.L}^{-1}$  could be attributed to crossing the pKa value for this surfactant ( $\text{pH} = 6.1$ ) (Ryu et al., 2013) at  $0.5 \text{ g.L}^{-1}$  thus favoring of acidic corrosion and (mixed) CuS dissolution combined with a reduction in surface tension or additional micellar effects of the biosurfactants which has been previously reported (Dhar et al., 2021; Ochoa-Loza et al., 2007).

Above  $5 \text{ g.L}^{-1}$  both target (Zn) and unwanted (Fe, Pb) elements continue to leach (Fig. 8-B and C), while Cu leaching reached a plateau, demonstrating that  $5 \text{ g.L}^{-1}$  is currently the optimal non-acetylated acidic sophorolipid concentration for leaching efficiency and selectivity. Given that the predicted  $\text{Cu}^{2+}$  solubility at pH 4.3 under the maximal Cu extraction concentrations of these experiments ( $1 \text{ mg.g}^{-1}$ ,  $1.6 \text{ mMol.L}^{-1}$ ) is high (SI Fig. 8-D), inhibition of leaching caused by formation of passive Cu layers ( $\text{CuO}$ ,  $\text{Cu(OH)}_2$ ) seems very unlikely. Similarly, passivation caused by sulfidic species after the dissolution of Cu is not expected as SEM imaging (Fig. 6) shows high leaching of Cu out of Cu-Pb sulfides after 7 days. Sudden passivation caused by strong biosurfactant-fayalite bonds also seems unlikely as the adsorption of this surfactant onto the fayalite matrix is in agreement with a linear adsorption isotherm (Dhar et al., 2019) (Fig. 8-D). Collectively, these results highlight that copper leaching in the fayalite was not limited by pH and biosurfactant concentration for Cu(0) oxidation and mixed CuS dissolution corrosion, thus a limitation on extractable CuO and CuS phases. Rather, Cu may be



**Fig. 6.** SEM imaging and corresponding EDX mapping on (A) starting material, (B) early leaching (0.5 h) and (C) long leaching (7d) of the fayalite with the non-acetylated acidic sophorolipid. Cu-Pb sulfides have been encircled.

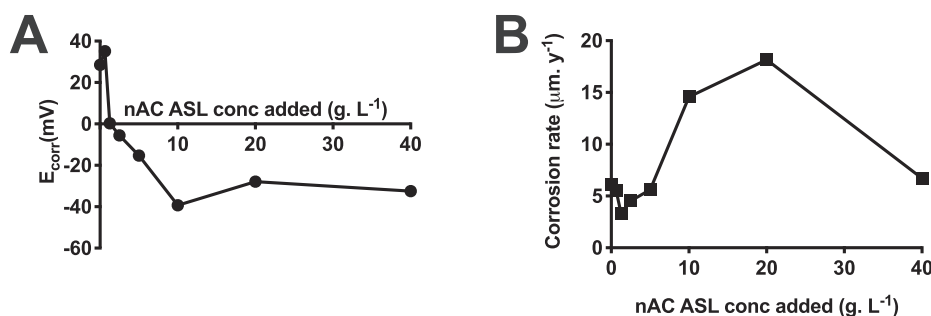
associated with fayalite as cuprospinel phases, as seen homogeneously distributed across the sample during the SEM imaging (Fig. 6-A).

#### 4. Conclusions and further work

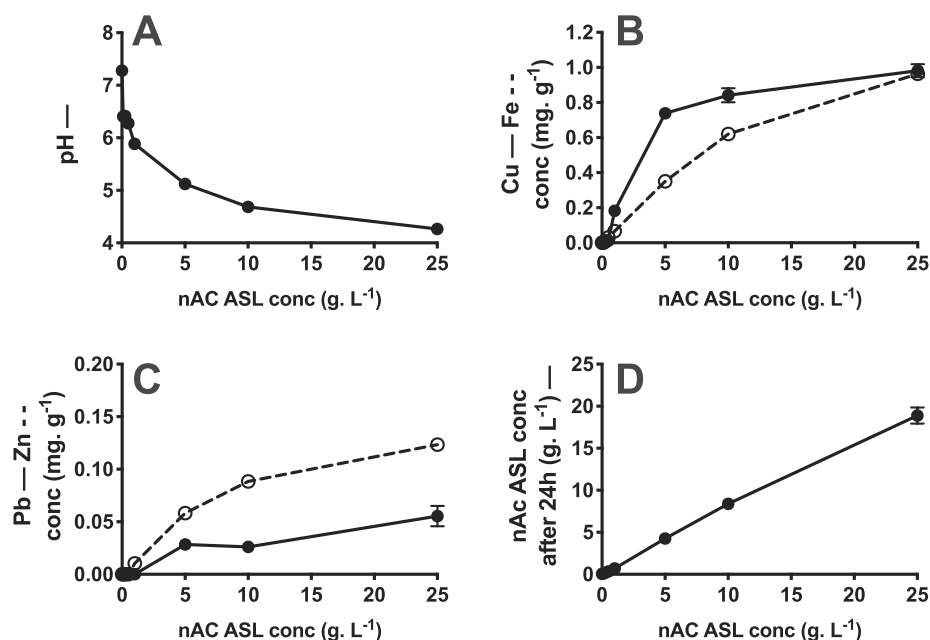
This study highlights the potential for biosurfactants, especially acidic sophorolipids, as an alternate and more sustainable technology for metal leaching from secondary materials. Specifically, we show that this technology is most performant for lower grade Cu(0) or CuS bearing

materials, which are likely proceeding via acidification and oxidation pathways. The additional performance of biosurfactants over pH effects may be associated with micellar stabilization and clearly warrants further investigation. CuS mineral phases are notoriously difficult to leach, thus biosurfactants provide a promising solution towards the abundant low grade tailing stockpiles worldwide. For higher grade materials bearing Cu(0), such as automotive shredder residues, biosurfactants are currently not as effective due to the insolubility of CuO towards neutral pH. Combining biosurfactants with other acidic (bio)





**Fig. 7.** Corrosion potential (A) and corrosion rate (B) determined from Tafel plots (SI Fig. 11). The increasing concentration of non-acetylated acidic sophorolipid shows the susceptibility of metallic Cu(0) towards corrosion.



**Fig. 8.** pH (A), leaching of Cu, Fe (B), Pb and Zn (C) and surfactant solubility (D) at different concentrations of the acidic non-acetylated sophorolipid on the fayalite material. The average value and standard deviation of  $N = 3$  replicates is plotted.

leaching technologies could thus have important synergistic leaching effects and warrants further work.

Future studies should focus on validating these observations with other pure mineral and alternative primary and secondary materials. Post leaching steps also need to be considered, including both the selective recovery of metals and the reusability of these biosurfactants to reduce costs and improve sustainability. In the case of acidic sophorolipids with carboxylic functionality, electrodialysis separation of the lixiviant and metals could be explored, similar to a previous study using citric acid (Maes et al., 2017). Overall, this study provides important fundamental biosurfactant-metal and mineral interactions, demonstrates the potential for biosurfactants to leach metals from low grade secondary materials and provides important steps towards a circular and biobased economy.

#### CRediT authorship contribution statement

**Martijn Castelein:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Florian Verbruggen:** Investigation, Validation, Writing - review & editing. **Lisa Renterghem:** Conceptualization, Validation, Supervision. **Jeroen Spooren:** Investigation, Writing - review & editing. **Lourdes Yurramendi:** Investigation. **Gijs Du Laing:** Resources, Writing - review

& editing. **Nico Boon:** Resources, Writing - review & editing. **Wim Soetaert:** Resources. **Tom Hennebel:** Writing - review & editing. **Sophie Roelants:** Resources, Writing - review & editing. **Adam Williamson:** Supervision, Project administration, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

The authors thank Bio Base Europe Pilot plant for supplying the biosurfactants that enabled the execution of the leaching experiments. We also thank Joachim Neri, Karel Folens, Nina Ricci Nicomel and Melgü Kizilmeşe for their assistance during ICP-analyses.

#### Funding

This work was supported by the SIM-SBO SMART (HBC.2016.0456)

and H2020 METGROW (690088) Grants, the European FP7 Project Biosurfing [nr. 289219]; European Horizon 2020 Bio-Based Industries (BBI) Consortium Project Carbosurf [nr. 669003]; and the Flemish Vlaio VIS project APPLISURF [HBC.2017.0704]. FV acknowledges support by the Flemish Agency for Innovation and Entrepreneurship (Vlaio) via a Baekeland PhD fellowship (HBC.2017.0224) and by the Research & Development Umicore Group.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mineng.2020.106665>.

## References

- Rietveld, E.; Boonman, H.; Harmelen, T. van; Hauck, M.; Bastein, T., 2018. Global Energy Transition And Metal Demand- An Introduction and Circular Economy Perspectives. 10.13140/RG.2.2.25790.54086.
- Guo, Y., Huang, C., Zhang, H., Dong, Q., 2009. Heavy Metal Contamination from Electronic Waste Recycling at Guiyu. Southeastern China. J. Environ. Qual. 38 (4), 1617–1626. <https://doi.org/10.2134/jeq2008.0398>.
- Weber, D. J.; Rutula, W. A., 2001. Use of Metals as Microbicides in Preventing Infections in Healthcare. In: Disinfection, Sterilization, and Preservation; Block, S. S., Ed.; Lippincott Williams and Wilkins, a Wolters Kluwer company: Philadelphia.
- Nessa, F., Khan, S., Abu shawish, K.Y.I., 2016. Lead, Cadmium and Nickel Contents of Some Medicinal Agents. Indian J. Pharm. Sci. 78 (1), 111. <https://doi.org/10.4103/0250-474X.180260>.
- Sethurajan, M., van Hullebusch, E.D., 2019. Leaching and Selective Recovery of Cu from Printed Circuit Boards. Metals (Basel) 9 (10), 1–12. <https://doi.org/10.3390/met9101034>.
- Desjardins, J., 2019. A Forecast of When We'll Run Out of Each Metal <http://www.visualcapitalist.com/forecast-when-we'll-run-out-of-each-metal/> (accessed Sep 3, 2019).
- Arda, C., Blowes, D.W., Ptacek, C.J., 2009. Comparison of Laboratory Testing Protocols to Field Observations of the Weathering of Sulfide-Bearing Mine Tailings. J. Geochemical Explor. 100 (2–3), 182–191. <https://doi.org/10.1016/j.geexplo.2008.06.005>.
- Sethurajan, M., van Hullebusch, E.D., Nancharaiya, Y.V., 2018. Biotechnology in the Management and Resource Recovery from Metal Bearing Solid Wastes: Recent Advances. J. Environ. Manage. 211, 138–153. <https://doi.org/10.1016/j.jenvman.2018.01.035>.
- Potysz, A., van Hullebusch, E.D., Kierczak, J., 2018. Perspectives Regarding the Use of Metallurgical Slags as Secondary Metal Resources – A Review of Bioleaching Approaches. J. Environ. Manage. 219, 138–152. <https://doi.org/10.1016/j.jenvman.2018.04.083>.
- Piatka, N.M., 2018. Environmental Characteristics and Utilization Potential of Metallurgical Slag. Environmental Geochemistry: Site Characterization, Data Analysis and Case Histories; Elsevier 487–519. <https://doi.org/10.1016/B978-0-444-63763-5.00020-3>.
- Alloway, B. J., 2012. Sources of Heavy Metals and Metalloids in Soils. In: Heavy metals in soils - Trace Metals and Metalloids in Soils and their Bioavailability (Environmental Pollution book series); Alloway, B. J., Ed.; Springer, Dordrecht, pp. 11–50. 10.1007/978-94-007-4470-7\_2.
- Kurylak, W., Mroueh, U.-M., Casanovas, S., Barras Garcia, R., 2016. Mapping the Secondary Resources in the EU. (Urban Mines) 688993, 78.
- Garside, M., 2019. Copper - Statistics & Facts <https://www.statista.com/topics/1409/copper/> (accessed Dec 8, 2019).
- Gorai, B., Jana, R.K., Premchand, 2003. Characteristics and Utilisation of Copper Slag—a Review. Resour. Conserv. Recycl. 39 (4), 299–313. [https://doi.org/10.1016/S0921-3449\(02\)00171-4](https://doi.org/10.1016/S0921-3449(02)00171-4).
- Sudbury, M., 2009. Smelter Slag - Seeking Market Opportunities & Carbon Credits and in a Changing World. In: Sánchez, M., Parra, R., Riveros, G., Díaz, C. (Eds.), VIII international conference on molten slags, fluxes and salts. Santiago, Chile, Gecamin Ltd, pp. 621–628.
- Hunt, A.; Clark, J. H.; Kraus, G. A.; Stankiewicz, A.; Van Gerven, T.; Abbott, A. P.; Li, S. F. Y.; Anderson, C.; Natrajan, L., 2013. Integration of Traditional Methods for Elemental Recovery in Zero-Waste Recycling Flow Sheet (Metal Recovery from Metallurgical Waste). Element Recovery and Sustainability (RSC Green chemistry series); Hunt, A., Ed.; Royal Society of Chemistry, 38–53.
- Bouzayani, F., Aydi, A., Abichou, T., 2014. Soil Contamination by Heavy Metals in Landfills: Measurements from an Unlined Leachate Storage Basin. Environ. Monit. Assess. 186 (8), 5033–5040. <https://doi.org/10.1007/s10661-014-3757-y>.
- Ettler, V., 2016. Soil Contamination near Non-Ferrous Metal Smelters: A Review. Appl. Geochemistry 64, 56–74. <https://doi.org/10.1016/j.apgeochem.2015.09.020>.
- Michaud, D., 2019 Flotation reagents <https://www.911metallurgist.com/blog/flotation-reagents> (accessed Apr 17, 2019).
- Hoque, M.E., Philip, O.J., 2011. Biotechnological Recovery of Heavy Metals from Secondary Sources-An Overview. Mater. Sci. Eng. C 31 (2), 57–66. <https://doi.org/10.1016/j.msec.2010.09.019>.
- Mujicic, V.; Coleman, K.; O'brien, K., 2016. Leach Aid for Metal Recovery. WO2016164600A1.
- Seelmann-eggebert, H.-P.; Oetter, G.; Berastain, A.; Ostos Rios, C., 2006. Use of Non-Ionic Surfactants in the Production of Metals. WO2006069738A1.
- Muravyov, M. I.; Pomchenko, N. V.; Usoltsev, A. V.; Vasilyev, E. A.; Kondrat'eva, T. F., 2012. Leaching of Copper and Zinc from Copper Converter Slag Flotation Tailings Using H2SO4 and Biologically Generated Fe2(SO4)3. Hydrometallurgy, 119–120, 40–46. 10.1016/j.hydromet.2012.03.001.
- Johnson, D.B., 2014. Biomining—Biotechnologies for Extracting and Recovering Metals from Ores and Waste Materials. Curr. Opin. Biotechnol. 30, 24–31. <https://doi.org/10.1016/j.copbio.2014.04.008>.
- Natarajan, K.A., 2018. Extended Applications of Metals Biotechnology. Biotechnol. Met. 305–353. <https://doi.org/10.1016/B978-0-12-804022-5.00011-6>.
- Pollmann, K., Kutschke, S., Matys, S., Raff, J., Hlawacek, G., Lederer, F.L., 2018. Bio-Recycling of Metals: Recycling of Technical Products Using Biological Applications. Biotechnol. Adv. 36 (4), 1048–1062. <https://doi.org/10.1016/j.biotechadv.2018.03.006>.
- Grand View Research, 2018. Global Biosurfactants Market By Product <https://www.grandviewresearch.com/press-release/global-biosurfactants-market> (accessed Aug 26, 2018).
- Markets and markets, 2017. Biosurfactants Market by Type (Glycolipids (Sphorolipids, Rhamnolipids), Lipopeptides, Phospholipids, Olymeric Biosurfactants), Application (Detergents, Personal Care, Agricultural Chemicals, Food Processing), and Region - Global Forecast to 2022. p. 116.
- Van Renterghem, L., Maes, K., Everaert, B., Redant, E., Vanlerberghe, B., De Maeseneire, S.L., Soetaert, W., 2018. Taking Biosurfactants from the Lab to the Market: Hurdles and How to Take Them by Applying an Integrated Process Design Approach. Microbial Biosurfactants and their Environmental and Industrial Applications.
- Schippers, C., Geßner, K., Müller, T., Scheper, T., 2000. Microbial Degradation of Phenanthrene by Addition of a Sphorolipid Mixture. J. Biotechnol. 83 (3), 189–198. [https://doi.org/10.1016/S0168-1656\(00\)00304-7](https://doi.org/10.1016/S0168-1656(00)00304-7).
- Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., Nowack, B., 2004. Extraction of Heavy Metals from Soils Using Biodegradable Chelating Agents. Environ. Sci. Technol. 38 (3), 937–944. <https://doi.org/10.1021/es0348750>.
- Kim, E.J., Jeon, E.K., Baek, K., 2016. Role of Reducing Agent in Extraction of Arsenic and Heavy Metals from Soils by Use of EDTA. Chemosphere 152, 274–283. <https://doi.org/10.1016/j.chemosphere.2016.03.005>.
- Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Heavy Metal Removal from Sediments by Biosurfactants. J. Hazard. Mater. 85 (1–2), 111–125. [https://doi.org/10.1016/S0304-3894\(01\)00224-2](https://doi.org/10.1016/S0304-3894(01)00224-2).
- Yang, Z., Zhang, Z., Chai, L., Wang, Y., Liu, Y., Xiao, R., 2016. Bioleaching Remediation of Heavy Metal-Contaminated Soils Using Burkholderia Sp. Z-90. J. Hazard. Mater. 301, 145–152. <https://doi.org/10.1016/j.jhazmat.2015.08.047>.
- Mulligan, C.N., Wang, S., 2006. Remediation of a Heavy Metal-Contaminated Soil by a Rhamnolipid Foam. Eng. Geol. 85 (1–2), 75–81. <https://doi.org/10.1016/j.enggeo.2005.09.029>.
- Massara, H., Mulligan, C.N., Hadjinicolaou, J., 2007. Effect of Rhamnolipids on Chromium-Contaminated Kaolinite. Soil Sediment Contam. 16 (1), 1–14. <https://doi.org/10.1080/15320380601071241>.
- Aşçı, Y., Nurbaz, M., Sağ Açıkel, Y., 2010. Investigation of Sorption/Desorption Equilibria of Heavy Metal Ions on/from Quartz Using Rhamnolipid Biosurfactant. J. Environ. Manage. 91 (3), 724–731. <https://doi.org/10.1016/j.jenvman.2009.09.036>.
- Dahrazma, B., Mulligan, C.N., 2007. Investigation of the Removal of Heavy Metals from Sediments Using Rhamnolipid in a Continuous Flow Configuration. Chemosphere 69 (5), 705–711. <https://doi.org/10.1016/j.chemosphere.2007.05.037>.
- Franzetti, A.; Gandolfi, I.; Fracchia, L.; Hamme, J. Van; Gkorezis, P.; Marchant, R., 2014. Biosurfactant Use in Heavy Metal Removal from Industrial Effluents and Contaminated Sites. In: Biosurfactants: Production and Utilization—Processes, Technologies, and Economics; Kosaric, N. F. V. S., Ed.; CRC Press: Boca Raton, FL, USA, pp. 361–370.
- Diaz, J.M., Hansel, C.M., Voelker, B.M., Mendes, C.M., Andeer, P.F., Zhang, T., 2013. Widespread Production of Extracellular Superoxide by Heterotrophic Bacteria. Science 340 (6137), 1223–1226. <https://doi.org/10.1126/science.1237331>.
- Gorin, P.A.J., Spencer, J.F.T., Tulloch, A.P., 1961. Hydroxy Fatty Acid Glycosides of Sophorose From Torulopsis Magnoliae. Can. J. Chem. 39 (4), 846–855. <https://doi.org/10.1139/v61-104>.
- Van Renterghem, L.; Roelants, S.; Remmery, J.; Mincke, S.; Christiaens, Q.; Hartmann, M.; Stevens, C.; Soetaert, W., 2020. Production of Novel Glycosides by Starmerella Bombicola (Manuscript Submitted for Publication).
- Price, N.P.J., Ray, K.J., Vermillion, K.E., Dunlap, C.A., Kurtzman, C.P., 2012. Structural Characterization of Novel Sphorolipid Biosurfactants from a Newly Identified Species of Candida Yeast. Carbohydr. Res. 348, 33–41. <https://doi.org/10.1016/j.carres.2011.07.016>.
- Van Renterghem, L., Roelants, S.L.K.W., Baccile, N., Uyttersprot, K., Taelman, M.C., Everaert, B., Mincke, S., Ledegen, S., Debrouwer, S., Scholtens, K., et al., 2018. From Lab to Market: An Integrated Bioprocess Design Approach for New-to-Nature Biosurfactants Produced by Starmerella Bombicola. Biotechnol. Bioeng. 115 (5), 1195–1206. <https://doi.org/10.1002/bit.26539>.
- Fuhrhop, J.-H., Wang, T., 2004. Bolaamphiphiles. Chem. Rev. 104 (6), 2901–2938. <https://doi.org/10.1021/cr030602b>.
- Baccile, N., Cuvier, A.S., Prévost, S., Stevens, C.V., Delbeke, E., Berton, J., Soetaert, W., Van Bogaert, I.N.A., Roelants, S., 2016a. Self-Assembly Mechanism of PH-Responsive Glycolipids: Micelles, Fibers, Vesicles, and Bilayers. Langmuir 32 (42), 10881–10894. <https://doi.org/10.1021/acs.langmuir.6b02337>.

- Baccile, N., Babonneau, F., Jestin, J., Pehau-Arnaudet, G., Van Bogaert, I., 2012. Unusual, PH-Induced, Self-Assembly of Sophorolipid Biosurfactants. *ACS Nano* 6 (6), 4763–4776. <https://doi.org/10.1021/nn204911k>.
- Puri, A., Loomis, K., Smith, B., Lee, J.-H., Yavlovich, A., Heldman, E., Blumenthal, R., 2009. Lipid-Based Nanoparticles as Pharmaceutical Drug Carriers: From Concepts to Clinic. *Crit. Rev. Ther. Drug Carrier Syst.* 26 (6), 523–580.
- Van Renterghem, L., Guzzetta, F., Le Griel, P., Selmane, M.; Messaoud, G. Ben; Tan Su Teng, T.; Lim, S.; Soetaert, W.; Roelants, S.; Julián-López, B.; et al., 2019. Easy Formation of Functional Liposomes in Water Using a PH-Responsive Microbial Glycolipid: Encapsulation of Magnetic and Upconverting Nanoparticles. *ChemNanoMat* 5 (9), 1188–1201. [10.1002/cnma.201900318](https://doi.org/10.1002/cnma.201900318).
- Baccile, N., Delbeke, E.I.P., Brennich, M., Seyrig, C., Everaert, J., Roelants, S.L.K.W., Soetaert, W., Van Bogaert, I.N.A., Van Geem, K.M., Stevens, C.V., 2019. Asymmetrical, Symmetrical, Divalent, and Y-Shaped (Bola)Amphiphiles: The Relationship between the Molecular Structure and Self-Assembly in Amino Derivatives of Sophorolipid Biosurfactants. *J. Phys. Chem. B* 123 (17), 3841–3858. <https://doi.org/10.1021/acs.jpcc.9b01013>.
- Dhasaiyan, P., Prevost, S., Baccile, N., Prasad, B.L.V., 2018. PH- and Time-Resolved in Situ SAXS Study of Self-Assembled Twisted Ribbons Formed by Elaidic Acid Sophorolipids. *Langmuir* 34 (5), 2121–2131. <https://doi.org/10.1021/acs.langmuir.7b03164>.
- Baccile, N., Le Griel, P., Prevost, S., Everaert, B., van Bogaert, I.N.A., Roelants, S., Soetaert, W., 2017. Glucosomes: Glycosylated Vesicle-in-Vesicle Aggregates in Water from PH-Responsive Microbial Glycolipid. *ChemistryOpen* 6 (4), 526–533. <https://doi.org/10.1002/open.201700101>.
- Peyre, J., Hamraoui, A., Faustini, M., Humblot, V., Baccile, N., 2017. Surface-Induced Assembly of Sophorolipids. *Phys. Chem. Chem. Phys.* 19 (23), 15227–15238. <https://doi.org/10.1039/C7CP01339F>.
- Dhasaiyan, P., Le Griel, P., Roelants, S., Redant, E., Van Bogaert, I.N.A., Prevost, S., Prasad, B.L.V., Baccile, N., 2017. Micelles versus Ribbons: How Congeners Drive the Self-Assembly of Acidic Sophorolipid Biosurfactants. *ChemPhysChem* 18 (6), 643–652. <https://doi.org/10.1002/cphc.201601323>.
- Baccile, N., Selmane, M., Le Griel, P., Prevost, S., Perez, J., Stevens, C.V., Delbeke, E., Zibek, S., Guenther, M., Soetaert, W., et al., 2016b. PH-Driven Self-Assembly of Acidic Microbial Glycolipids. *Langmuir* 32 (25), 6343–6359. <https://doi.org/10.1021/acs.langmuir.6b00488>.
- Sarubbo, L.A., Rocha, R.B., Luna, J.M., Rufino, R.D., Santos, V.A., Banat, I.M., 2015. Some Aspects of Heavy Metals Contamination Remediation and Role of Biosurfactants. *Chem. Ecol.* 7540 (November), 1–17. <https://doi.org/10.1080/02757540.2015.1095293>.
- Rufino, R.D., Luna, J.M., Campos-Takaki, G.M., Ferreira, S.R.M., Sarubbo, L.A., 2012. Application of the Biosurfactant Produced by *Candida Lipolytica* in the Remediation of Heavy Metals. *Chem. Eng. Trans.* 27 (Gomes 2000), 61–66. doi: 10.3303/CET1227011.
- Luna, J.M., Rufino, R.D., Sarubbo, L.A., 2016. Biosurfactant from *Candida Sphaerica* UCP0995 Exhibiting Heavy Metal Remediation Properties. *Process Saf. Environ. Prot.* 102, 558–566. <https://doi.org/10.1016/j.psep.2016.05.010>.
- Mulligan, C.N., Yong, R.N., Gibbs, B.F., James, S., Bennett, H.P.J., 1999. Metal Removal from Contaminated Soil and Sediments by the Biosurfactant Surfactin. *Environ. Sci. Technol.* 33 (21), 3812–3820. <https://doi.org/10.1021/es9813055>.
- Williamson, A.J., Verbruggen, F., Chavez Rico, V.S., Bergmans, J., Sporeen, J., Yurramendi, L., Laing, G.Du., Boon, N., Hennebel, T., 2021. Selective Leaching of Copper and Zinc from Primary Ores and Secondary Mineral Residues Using Biogenic Ammonia. *J. Hazard. Mater.* 403, 123842. <https://doi.org/10.1016/j.jhazmat.2020.123842>.
- Aldrian, A., Raith, J.G., Höllen, D., Pomberger, R., 2015. Influence of Chromium Containing Spinel in an Electric Arc Furnace Slag on the Leaching Behaviour. *J. Solid Waste Technol. Manag.* 41 (4), 357–365. <https://doi.org/10.5276/JSWTM.2015.357>.
- Mardones, J.P., 2012. Processing of Spinel-Bearing Compounds for Zinc Extraction. Delft University of Technology.
- Dhar, P., Havskjold, H., Thornhill, M., Roelants, S., Soetaert, W., Kota, H.R., Chernyshova, I.V., 2021. Toward Green Flotation: Interaction of a Sophorolipid Biosurfactant with a Copper Sulfide. *J. Clean. Prod.* (in press).
- Parsons, M.B., Bird, D.K., Einaudi, M.T., Alpers, C.N., 2001. Geochemical and Mineralogical Controls on Trace Element Release from the Penn Mine Base-Metal Slag Dump. California. *Appl. Geochemistry* 16 (14), 1567–1593. [https://doi.org/10.1016/S0883-2927\(01\)00032-4](https://doi.org/10.1016/S0883-2927(01)00032-4).
- Zhang, R., Sun, C., Kou, J., Zhao, H., Wei, D., Xing, Y., 2018. Enhancing the Leaching of Chalcopyrite Using Acidithiobacillus Ferrooxidans under the Induction of Surfactant Triton X-100. *Minerals* 9 (1), 11. <https://doi.org/10.3390/min9010011>.
- Ram, R., Beiza, L., Becker, M., Pownceby, M.I., Chen, M., Yang, Y., Yang, S., Petersen, J., 2020. Study of the Leaching and Pore Evolution in Large Particles of a Sulfide Ore. *Hydrometallurgy* 192, 105261. <https://doi.org/10.1016/j.hydromet.2020.105261>.
- Jorjani, E., Ghahreman, A., 2017. Challenges with Elemental Sulfur Removal during the Leaching of Copper and Zinc Sulfides, and from the Residues; a Review. *Hydrometallurgy* 171, 333–343. <https://doi.org/10.1016/j.hydromet.2017.06.011>.
- Liu, W., Yang, H., Song, Y., Tong, L., 2015. Catalytic Effects of Activated Carbon and Surfactants on Bioleaching of Cobalt Ore. *Hydrometallurgy* 152, 69–75. <https://doi.org/10.1016/j.hydromet.2014.12.010>.
- Malik, M. A.; Hashim, M. A.; Nabi, F.; AL-Thabaiti, S. A.; Khan, Z., 2011. Anti-Corrosion Ability of Surfactants: A Review. *Int. J. Electrochem. Sci.* 6(6), 1927–1948.
- Maes, S., Zhuang, W.Q., Rabaey, K., Alvarez-Cohen, L., Hennebel, T., 2017. Concomitant Leaching and Electrochemical Extraction of Rare Earth Elements from Monazite. *Environ. Sci. Technol.* 51 (3), 1654–1661. <https://doi.org/10.1021/acs.est.6b03675>.
- Ryu, M. K.; Ito, H. K.; Araki, M. K., 2013. High-Purity Acid-Form Sophorolipid (SI) Containing Composition and Process for Preparing Same. WO2013129667A1.
- Ochoa-Loza, F.J., Noordman, W.H., Janssen, D.B., Brusseau, M.L., Maier, R.M., 2007. Effect of Clays, Metal Oxides, and Organic Matter on Rhamnolipid Biosurfactant Sorption by Soil. *Chemosphere* 66 (9), 1634–1642. <https://doi.org/10.1016/j.chemosphere.2006.07.068>.
- Dhar, P., Chernyshova, I.V., Thornhill, M., Roelants, S., Soetaert, W., Kota, H.R., 2019. Floatability of Chalcopyrite by Glycolipid Biosurfactants as Compared to Traditional Thiol Surfactants. *Tenside Surfactants Deterg.* 56 (5), 429–435. <https://doi.org/10.3139/113.110639>.
- Solaiman, D.K.Y., Roelants, S.L.K.W., Ashby, R., Van Renterghem, L., Lodens, S., Soetaert, W., 2018. Chapter 3: Production and Application of Sophorolipids. *Biobased surfactants Synth. Prop. Appl. Second Ed.* 84.